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Excretion patterns of solute and different-sized particle passage markers in foregut-fermenting proboscis monkey (*Nasalis larvatus*) do not indicate an adaptation for rumination

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Running head: Passage marker excretion in proboscis monkey

Abstract

Behavioral observations and small fecal particles compared to other primates indicate that free-ranging proboscis monkeys (*Nasalis larvatus*) have a strategy of facultative merycism (rumination). In functional ruminants (ruminant and camelids), rumination is facilitated by a particle sorting mechanism in the forestomach that selectively retains larger particles and subjects them to repeated mastication. Using a set of a solute and three particle markers of different sizes (<2, 5 and 8 mm), we displayed digesta passage kinetics and measured mean retention times (MRTs) in four captive proboscis monkeys (6–18 kg) and compared the marker excretion patterns to those in domestic cattle. In addition, we evaluated various methods of calculating and displaying passage characteristics. The mean ± SD dry matter intake was 98 ± 22 g $kg^{-0.75}$ d^{-1}, 68 ± 7% of which was browse. Accounting for sampling intervals in MRT calculation yielded results that were not affected by the sampling frequency. Displaying marker excretion patterns using fecal marker concentrations (rather than amounts) facilitated comparisons with reactor theory outputs and indicated that both proboscis and cattle digestive tracts represent a series of very few tank reactors. However, the separation of the solute and particle marker and the different-sized particle markers, evident in cattle, did not occur in proboscis monkeys, in which all markers moved together, at MRTs of approximately 40 h. The results indicate that the digestive physiology of proboscis monkeys does not show typical characteristics of ruminants, which may explain why merycism is only a facultative strategy in this species.

Key words: colobine, rumen, chemical reactor, anatomy, digestion

1. Introduction

Digesta retention and digesta flow are important elements of the digestive physiology for several reasons. The sheer time that digesta is retained in the digestive tract and thus subjected to processes of auto-enzymatic digestion and, in particular, allo-enzymatic digestion by a symbiotic gut microbiome [1], is a major determinant of the thoroughness of digestion. Because microbial digestion is particularly important for herbivores, they have comparatively long digesta retention times across a large variety of body sizes [2]. In addition, differences in the flow kinetics between different digesta phases can indicate relevant physiological processes. Examples are the retrograde washing of digesta in the hindgut of lagomorphs, which ensures that microbes are retained in the caecum, or the forward washing of forestomach contents in ruminants, which allows efficient harvesting of microbes growing in that compartment [3]. Another typical example is the particle sorting mechanism in the forestomach of ruminants, which ensures that larger particles are retained for a longer period of time and intermittently subjected to the process of rumination [4, 5].

The proboscis monkey (*Nasalis larvatus*), a member of the Old World monkey subfamily Colobinae, is a foregut fermenter [6, 7] that consumes natural diets with varying proportions of leaves and (mostly unripe) fruits and seeds [8-10]. Similar to other colobines, long digesta retention times were measured in captive specimens of this species [11, 12]. Free-ranging specimens were observed to perform a behavior indicating regurgitation and remastication of forestomach contents, suggestive of a 'rumination' strategy [13]. Compared with other primates, the particularly fine fecal particles in free-ranging proboscis monkeys support the overall concept of repeated mastication in this species [14]. Therefore, a detailed description of the flow of digesta components through the gastrointestinal tract (GIT) of this species is of interest.

First, one may wonder whether the proboscis monkey is an outlier to the general condition of primates that apparently do not achieve a difference in the kinetics of solutes and

74 particles in their GIT [3]. However, although no such difference is evident in the so-called
75 'moose-type' ruminants, these animals nevertheless achieve efficient particle size sorting.
76 Therefore, this difference need not be considered as an obligatory precondition for rumination
77 [15]. Second, if rumination in this species was convergent to true ruminants to a higher degree
78 than the sheer fact of repeated mastication, one would expect a similar sorting mechanism as
79 that observed in ruminants, with a pronounced longer delay of larger particles than smaller
80 particles [15]. Therefore, the aim of the present study was to measure the kinetics of passage
81 through the digestive tract of proboscis monkeys for different markers representing the
82 various digesta components. We report results from passage experiments in four individual
83 proboscis monkeys to which four different markers were simultaneously fed. We compared
84 the resulting excretion curves directly with that obtained in a domestic heifer and
85 demonstrated the effect of using different means of displaying the resulting marker excretion
86 patterns.
87

88 ## 2. Methods

89 *2.1 Study animals and diet*

90 In April 2014, the measurements were performed with three proboscis monkeys housed
91 together (animals 1-3; adult male: 24.0 years old with 18.0 kg of body mass; adult female 1:
92 10.3 yr with 8.0 kg; adult female 2: 7.8 yr with 8.0 kg) and one subadult female housed alone
93 (animal 4; 3.7 yr with 6.0 kg) at the Primate Holding, an off-exhibit facility area of the
94 Singapore Zoo [16]. The animals were fed a mixed diet of fresh leaves and vegetables four
95 times daily at 08:00, 11:00, 13:00 and 16:00. The staple leaf diet consisted of leaves from five
96 plant species in varying proportions: acalypha (*Acalypha siamensis*), hibiscus (*Hibiscus* sp.),
97 miracle (*Leucaena leucocephala*), mulberry (*Morus alba*) and ketapang (*Terminalia catappa*).
98 The vegetables consisted of a mix including sweet potatoes, long beans, French been, carrots
99 and sweet corn. All animals also received daily supplements of Mazuri® Primate Browse

pellets (Mazuri®, Indiana, USA). Water was freely available at all times. In order to be able to discriminate the fecal samples among three adults during the night, glass beads (1 mm diameter) of three different colors were fed in a small rice ball to each of the three individuals shortly before the first feeding (08:00) on a daily basis.

For a visual comparison of the same markers as excreted by a ruminant foregut fermenter, known to have sorting mechanism in its forestomach that results in a differential excretion of particles of different sizes [15], we used a heifer (domestic cattle, 320 kg, 13 months of age) kept in a tie-stall in Switzerland during an experiment which was approved by the veterinary office of the canton of Zurich (149/2013). The animal had previously been adapted to a diet of grass hay only, and was given grass hay *ad libitum* throughout the experiment.

2.2 Food consumption

Food consumption was recorded quantitatively over a period of eight consecutive days. Each food item was weighed before it was offered to the animals and left in their enclosures until the next feeding session. The mean (± standard deviation) daily amount of offered food per animal was, on an as fed basis, 4.1 ± 1.7 kg fresh leaves, 454 ± 28 g vegetables, 24 ± 4 g of primate pellets and 8 ± 23 g rice balls for the single sub-adult female, and 5.4 ± 0.5 kg fresh leaves, 572 ± 100 g vegetables, 32 ± 14 g of primate pellets, and 32 ± 37g rice balls for each of the three group-housed adults. Prior to the subsequent feeding sessions, all leftover food was removed and the enclosure cleaned before fresh food items were offered. All food items and leftovers were weighed with accuracy of 1 g (TERASEIKO Electronic Weighing Platform, Singapore). Leftover weights were adjusted by deriving a desiccation factor from the measured moisture lost from similar sets of food placed in a desiccation pan in an area adjacent to the primate enclosures. For the three adults housed together, individual food consumption was estimated by the ratio of the numbers of bites of each individual counted by

three observers throughout the entire day. For example, if 100 g of a diet item had disappeared between offering the item and taking out the leftovers (accounting for evaporation losses), and animal A had been observed to eat from this item in 8 bites, animal B in 2 bites and animal C not at all (i.e., a total of 10 bites), then it was assumed that animal A consumed 8/10 x 100 g = 80 g and animal B 2/10 x 100 g = 20 g of that item. In order to simulate the natural feeding behavior of proboscis monkeys that cease feeding during the night [8], no food was provided after 18:00. The dry matter (DM) concentration of representative samples of all feeds previously determined [17].

2.3 Passage markers, application, sampling and analysis

Cobalt (Co) was used as solute marker bound to EDTA [18]. As particle markers, mordanted fiber of different particle size was used, obtained from grass hay that was dried and coarsely cut in a cutting mill. The material was then dry screened to result in particle sizes of approximately 2, 5 and 8 mm, and submitted to washing in neutral detergent solution as prescribed for the method [18]. The three fractions were then mordanted in this order with chromium (Cr), lanthanum (La) and cerium (Ce), respectively, following the element-specific mordanting prescriptions outlined in previous studies [18, 19]. Marker concentration (in g per kg dry matter) in the Co-EDTA was 140 for Co, and in the mordanted material 38 for Cr, 16 for La and 13 for Ce. Co-EDTA was applied dissolved in water as a liquid. Particle sizes were chosen based on results from studies on a sorting mechanism in the forestomachs of different ruminants, where sorting could be demonstrated between particles of 2 mm and 10 mm, but not between particles of 10 mm and 20 mm [15, 19, 20]. All markers were fed as a pulse dose in the morning in rice balls, shortly before the first regular feeding (08:00) at 0.18-0.54 g Co-EDTA and 1.2-3.6 g of each fiber marker for the subadult female and the adult male, respectively (with other females receiving intermediate doses). For the subsequent 8 days, feces were collected between 06:00 and 18:00. During this time, each single defecation was

ascribed to an individual during constant observation, the time of the individual defecation was noted as well as its location in the enclosure, and the feces were collected from the enclosure at intervals of 2-3 hours (mostly shortly before feeding sessions). Feces voided between 18:00 and 06:00 were collected as individual defecations and ascribed to individuals according to the color of the marker beads. These night feces were pooled per individual, with the exception of the feces of the first night, when each defecation was collected individually. All fecal samples, which always represented the complete defecations, were dried immediately after collection to constant weight at 60°C, and their dry weight was registered. Samples were ground to pass through a 1mm sieve.

For the heifer, the Co-EDTA (dosage approximately 0.01 g per kg BW) and each mordanted fiber (dosage each approximately 0.1 g per kg BW) were formed to boluses and applied directly into the rumen using a commercial bolus applicator, and feces were collected at 4, 8, 12, 18, 22, 26, 30, 36, 42, 46, 52, 58, 66, 74, 82, 90, 98, 106, 114, 126, 138, 150 h after marker application.

The analysis of fecal samples for passage markers followed Frei, Ortmann [21]. After microwave wet ashing with nitric acid and hydrogen peroxide was performed, samples were submitted to inductively coupled plasma optical emission spectrometer (Optima 8000, Perkin Elmer, Rodgau, Germany), measuring spectral element lines at 228.616 nm (Co), 267.716 nm (Cr), 398.852 (La) and 413.764 nm (Ce).

2.4 Calculation of mean retention time

The mean retention time through the whole digestive tract (MRT) was calculated according to Thielemans, Francois [22] as

$$\mathrm{MRT} = \frac{\Sigma\, t_i\, C_i\, dt_i}{\Sigma\, C_i\, dt_i}$$

with C_i = marker concentration in the fecal samples from the interval represented by time after marker application t_i and dt_i = the interval (h) of the respective sample

$$dt_i = \frac{(t_{i+1}-t_i)+(t_i-t_{i-1})}{2}$$

Additionally, MRTs were calculated by an approach often used in primate studies [23-25] that was introduced for ruminants by Blaxter, Graham [26] and is mostly cited as promoted by Warner [27]. This approach uses the same equation for MRT as above but without dt_i in the numerator and the denominator. The marker was assumed to have been excreted completely once the fecal marker concentrations were similar to the background-levels determined in pre-dose fecal samples.

In order to control whether the results were influenced by the fact that in this experiment, a much more frequent fecal sampling was possible than usually performed in passage studies, two different assays to calculate MRT were used, basically repeating the test of Van Weyenberg, Sales [28]. In the first assay, t_i was defined as the exact time in case of individual defecations, or the midpoint of the sampling interval in the case of night samples; the time period of the first night was divided into as many time intervals as there were individual defecations per animal, and the order of the individual samples was defined subjectively by sorting the samples according to their marker concentrations. In the second assay, a sampling regime of fixed time intervals was assumed [as is common practice in passage studies, e.g. 15]. These intervals were every 4 h for the first two days, every 6 h for the third day, and every 8 h for the subsequent days; the night period was considered one sampling period for all nights; t_i was defined as the midpoint of each sampling interval. The marker concentrations for all feces that corresponded to a time interval were calculated using the dry weights and the marker concentrations of the individual samples (i.e., simulating a pooled sample).

199 *2.5 Visualization of marker excretion patterns*

200 Marker excretion was visualized by plotting marker concentrations in feces against time, as
201 raw data or by expressing the results in % of the peak concentration, in order to normalize the
202 different absolute concentrations. Additionally, to demonstrate the relevance of different ways
203 to display marker excretion curves, we also displayed the excretion pattern when the % of the
204 total marker dose was plotted against time, as done e.g. by Caton [25].

205

206 *2.6 Statistics*

207 Results were indicated as means ± standard deviation. Differences between markers, and
208 differences between assays, were evaluated using paired t-tests, correcting for multiple testing
209 with Sidak post hoc tests. Statistical analyses were performed with SPSS 21.0 (SPSS Inc.,
210 Chicago IL, USA), with the significance level set to $P < 0.05$, with values between 0.05 and
211 0.08 considered as trends.

212

213 ## 3. Results

214 The proboscis monkeys ingested on average 518 ± 123 g DM d^{-1} or 98 ± 22 g DM $kg^{-0.75}$ d^{-1}.
215 Browse represented 68.4 ± 6.6% of the total DM intake. The defecation frequency varied
216 from 21 to 31 times per day (Table 1), with the highest value observed in the solitary animal.
217 Irrespective of the sampling regime, MRT for all markers was approximately 40 h (Table 2)
218 when the equation of Thielemans, Francois [22] was used, with no significant differences
219 between sampling regimes for any marker (paired t-tests, $P = 0.547$-0.825). In addition, when
220 using paired t-tests with Sidak adjustment for multiple testing, there was no significant
221 difference between the different markers in either sampling regime. Using the equation of
222 Warner [27] resulted in MRTs of approximately 32 h for all markers in the intensive sampling
223 regime and approximately 37 h for the less frequent sampling regime (simulating pooled fecal
224 samples); this difference tended towards significance for Co ($P = 0.067$), La ($P = 0.066$) and

225 Ce (P = 0.060). Again, within each sampling regime, there was no significant difference
226 between the different markers. For both the sampling regimes, MRTs calculated according to
227 Warner [27] were significantly shorter by 3-8 h than those calculated according to
228 Thielemans, Francois [22] (intensive sampling: P = 0.002-0.003; less-frequent sampling: P =
229 0.011-0.030).
230 The marker excretion curves showed the general pattern of a fast increase and a
231 subsequent more gradual decline in marker concentrations (Fig. 1-2), typical of a few
232 continuously stirred tank reactors (CSTRs) in sequence (Fig. 3). Variation in the marker
233 concentration among subsequent defecations led to a 'noisy' excretion pattern with many
234 individual spikes and declines, which were smoothened when data were presented by larger
235 sampling intervals simulating pooled fecal samples (Fig. 2 left and right column). Secondary
236 marker excretion peaks following the first one were evident to a certain degree in all animals
237 and particularly prominent in animals 1 and 3.
238 When comparing marker excretion patterns in proboscis monkeys with those in the
239 domestic heifer using various methods of data visualization (Fig. 2), the most striking
240 difference was the clear separation of the solute and particle marker and the small vs. large
241 particle marker in the heifer. In contrast, all markers moved through the digestive tract in
242 unison in proboscis monkeys. When using absolute concentrations for evaluation, differences
243 between marker doses can lead to a visual pattern that suggests a larger difference between
244 markers (Fig. 2a) than that evident when marker concentrations are standardized by
245 expressing them as a proportion of the peak concentration (Fig. 2b). A closer view of the
246 marker excretion peak (Fig. 2c) makes it evident that the excretion curves are not as smooth
247 as predicted in ideal chemical reactors (Fig. 3). In particular, marker sequestration with
248 incomplete mixing is suggested for proboscis monkeys. Compared with the smoother
249 excretion curve in the heifer, displaying the data at larger sampling intervals emphasizes this
250 effect in proboscis monkeys (Fig. 2d). When expressing the results as a percentage of the total

marker dose, the curves of both proboscis monkeys and heifer have a very different appearance, with more exaggerated multiple spikes and a loss of the typical fast increase–gradual decrease shape evident in previous visualizations (Fig. 2e).

4. Discussion

The present study provides an instructive example of the consequences of choosing different algebraic and visualization methods for passage marker excretion data. For the model animal of the present study, the data indicate no deviation from the general colobine and primate pattern, which is characterized by the lack of separation of solute and particle marker excretion [3, 25, 29] and the absence of selective retention of larger particles in comparison with smaller particles [30].

4.1 Limitations of the present study

A typical constraint of investigations of the digestive physiology of non-domestic species is that experiments can mostly only be performed with captive individuals, which are exposed to unnatural conditions such as the solitary husbandry of animal 4 of the present study, which may have represented a stressful situation, resulting in particularly high defecation rates. Diets that do not correspond to the natural ones are very typical for digestion studies in captive primates, which receive various pelleted feeds, fruits, vegetables and starchy items grown for human consumption [31, 32]. In the present study, the high proportion of browse in the overall food intake (Table 1) most likely made the diet more similar to the natural diet of proboscis monkeys than that used during a previous experiment by Dierenfeld, Koontz [11]. This may also have contributed to the substantially higher food intake in the present study than in the previous study (98 vs. 32 g $kg^{-0.75}$ d^{-1}) and, concomitantly, the somewhat shorter MRT (40 vs. 49 h).

That previous study is also a good example of a typical constraint of zoo-based research (in comparison with procedures in experimental facilities explicitly destined for animal research), namely the frequency at which fecal samples can be collected, which is often determined by the routines of the keepers [e.g. 25]. Dierenfeld, Koontz [11] stated that in their proboscis study, feces were collected twice daily. The intensive observations during the present study enabled a much higher sampling frequency; however, these were also limited to a time period that did not extend the regular working hours of the keepers by a large margin. When measuring retention times, the degree to which the sampling frequency will influence the result is an important question, which we address further down below (cf. 4.2).

In addition, feeding regimes in captivity, with comparatively highly digestible feeds and comparatively low intakes, may result in reduced defecation frequencies. For example, Caton [25] observed that some individuals investigated did not defecate in the late afternoon (and, by implication, at night). In contrast, the individuals in the present study often defecated at night. Night-time defecations have been reported in free-ranging chimpanzees [33], and during night-time observations of free-ranging proboscis monkeys [34], defecation was frequently observed (I. Matsuda, pers. obs.). Because sampling outside the normal husbandry routine of entering enclosures for feeding or cleaning may represent additional stress, possibly triggering diarrhea or increased defecation rates (J. Caton, pers. obs.), it would have been ideal to record the time of night defecations by observations. However, although night vision recording of animal activities enabled behavioral observations, it was not feasible to time individual defecations by studying the recordings (I. Matsuda, pers. obs.).

Finally, we used only a single heifer for comparison; however, the resulting marker excretion pattern was as reported in other studies with functional ruminants [15, 20, 35], and corresponds to differences between the MRT of different-sized particles in various studies with ruminants [4, 36, 37].

4.2 Calculation of MRTs

Van Weyenberg, Sales [28] showed that MRT, as calculated on the basis of the equation of Blaxter, Graham [26] and mostly ascribed to Warner [27], depends on the sampling interval, becoming shorter with more frequent sampling. This was confirmed in the present study, where the much higher sampling frequency of the original approach led to MRTs of approximately 32 h, in contrast to 37 h observed in the approach using the same calculation with less frequent sampling intervals (simulating pooled samples). Van Weyenberg, Sales [28] also showed that including the sampling interval in the calculation, performed in the present study using the equation of Thielemans et al. (1978), makes the MRT estimation independent of the sampling frequency. Again, this finding was evident in our data. Therefore, we recommend the use of the latter approach for the future calculation of MRT.

4.3 Visualization of passage marker excretion patterns

In the attempt to understand animal digestive tracts, they have been linked to chemical reactor theory in two manners: by the similarity of digestive tract segments with individual reactor types [38, 39] and by the marker excretion patterns linked to certain series of reactor types [40, 41]. The visualization of marker excretion patterns is crucial for this comparison; however, there appears to be no consensus on the way in which marker excretion patterns are depicted in the literature. Chemical reactor models predict a marker flow pattern that is based on concentrations over time (Fig. 3). Some studies actually indicate the marker excretion pattern in terms of marker concentrations [e.g. 29, 30, 42, 43] using various untransformed or (usually log-) transformed scales [reviewed in 44]. This can be done by providing each marker in its true concentration [e.g. 29], which can lead to an optical separation of marker curves simply because of different dosage levels (cf. Fig. 2a). To avoid such an impression, marker units are either adjusted on multiple scales to achieve similar maxima [e.g. 30] or the concentration is expressed as a proportion of its maximum ('% of peak') [e.g. 15]. An

328 alternative way of displaying marker excretion patterns is to use the excreted amounts rather
329 than concentrations. In this approach, excretion is often expressed as a fraction of the total
330 dose (or the total amount excreted/recovered). This is done either in a cumulative manner
331 [e.g. 42, reviewed in 44] so that the excretion curve approximates 100% with time, or in a
332 non-cumulative manner. The display of non-cumulative marker excretion patterns as a
333 proportion of the total dose has often been used in primates, either for individual sampling
334 events [23, 25, 45, 46] or for defined intervals of equal length [11, 47]. The latter adjustment
335 is performed because the amount of excreted marker is dependent not only on the marker
336 concentration in the feces but also on the amount of feces defecated in the respective
337 sampling interval; differences in sampling interval lengths could therefore lead to a distortion
338 of the excretion patterns, simply because different amounts of feces are considered in
339 different intervals.

340 In Figure 2, we showed, in a primate and a ruminant, that the same dataset can yield
341 very different passage marker excretion curves, depending on the method of visualization.
342 Both the pattern typical for a small number of CSTRs (Fig. 2b-d) and a pattern that cannot be
343 reconciled with any hypothetical series of chemical reactors (Fig. 2e) could be produced,
344 yielding marker excretion curves for foregut fermenters as in Schwarm, Ortmann [30; cf. Fig.
345 2 b-d] or as in Caton [25; cf. Fig. 2e]. The statement of Caton [25] that colobine monkeys
346 have marker excretion patterns that are fundamentally different from those observed in
347 ruminants or macropods is based on a comparison of two different types of visualizations (i.e.
348 in a comparison of Fig. 2e for primates to Fig. 2b for ruminants/macropods). We recommend
349 that future comparisons should be based on marker excretion patterns that display the change
350 in the fecal marker concentration over time (i.e. as in Fig. 2b), as opposed to presenting the
351 proportion of the total amount recovered.

352

353 *4.4 Digestive physiology of the proboscis monkey*

Regardless of the difference in data display between the study by Caton [25] and the present study, some interpretations made in the previous study appear to hold true. In all four proboscis monkeys, uneven marker excretion patterns as well as secondary marker excretion peaks occurred (extremely prominent in animal 1 and 3 and less prominent but visible in animal 2 and 4). Usually, such secondary marker excretion peaks would be interpreted as an indication for re-ingestion of marker via coprophagy [44]. However, coprophagy was never observed during the experimental period in proboscis monkeys, and concomitant night-time observations using an infrared equipment for another study did not indicate the occurrence of coprophagy during times when observers were not present (I.M., pers. obs.). Therefore, we interpret these marker excretion spikes and secondary peaks as an indication that digesta is less thoroughly mixed in the forestomach of colobines than in the forestomach of ruminants and that the sequestration of digesta can occur either in the forestomach or in the caecum and colon. As observed by Chivers [7] and Caton [25], the hindgut of colobine monkeys is more pronounced than that of other mammalian foregut fermenters, both in terms of the length and volume and in terms of the macroscopic appearance with its taeniae and haustra (Fig. 4). If it had the effect of additional mixing chambers, one would expect the marker excretion curves of proboscis monkeys to show a more gradual increase at the beginning (Fig. 3). The comparison of the proboscis monkey and heifer in Fig. 2b may suggest a slightly lesser steep initial increase in the monkey than in the solute marker in the heifer; however, the effect is not particularly pronounced, which suggests that, digesta sequestration effects notwithstanding, the hindgut functions like a plug-flow reactor than like a series of CSTRs. It is remarkable that the initial stages of the excretion curves in the heifer differ for the solute and particle marker (Fig. 2b), suggesting a larger number of CSTRs for particles than for fluid, although both evidently move through the same digestive tract. This is in accordance with the interpretation that particles move through separate ‘pools’ in the forestomach of ruminants

because of particle separation mechanisms and intermittent rumination and changes in size they are exposed to [e.g. 48].

Although behavioral observations [13] and measurements of the fecal particle size [14] suggest regurgitation and remastication (merycism) to be a facultative part of the digestive strategy of proboscis monkeys, the data of the present study indicate that the forestomach of this species is not adapted to a selective retention of particles of a specific size. Such a mechanism is the major characteristic of the forestomach of ruminants or camelids [49] but is absent in non-ruminant foregut fermenters [20, 30]. Merycism has also been observed in other species without a known particle separation mechanism in the (fore)stomach, such as macropods [50, 51] and koalas (*Phascolarctos cinereus*) [52]. Compared with other non-ruminant (non-primate) foregut fermenters, particle size has been found to be comparatively fine in macropods [53]. In koalas, merycism can compensate for the effect of tooth wear and facilitate higher food intakes [54, 55]. The same was indicated by feeding observations in a single proboscis monkey that spent a longer time feeding on days during which merycism was observed than during days when it was not observed [13]. A particle sorting mechanism in the (fore)stomach therefore need not be considered to be a prerogative for the strategy of merycism but can be interpreted as the hallmark of the ruminating foregut fermenters that truly sets them apart from other mammalian herbivores [53].

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565 **Table 1** Animals, intake, defecations and mean retention times (MRTs)*, as calculated on the
566 basis of the intensive sampling frequencies for four different passage markers in proboscis
567 monkeys (*Nasalis larvatus*)
568

Animal (sex)	Body mass	DMI	rDMI	Browse	Defecations	---------- MRT* intensive sampling ----------			
						Co (solute)	Cr (2mm)	La (5mm)	Ce (8mm)
	kg	g d^{-1}	g $kg^{-0.75}$ d^{-1}	%DMI	n d^{-1}	h			
1 (m)	18.0	645	74	66.2	21.0	47.8	46.8	46.9	46.0
2 (f)	8.0	598	126	75.0	24.1	38.1	38.9	37.7	37.3
3 (f)	8.0	436	92	60.2	21.3	43.0	44.2	42.1	41.6
4 (f)	6.0	392	102	72.1	31.1	35.8	34.9	34.6	34.8

569 DMI dry matter intake, rDMI relative dry matter intake; apart from browse, various vegetables and a pelleted primate diet
570 were fed
571 *calculated according to Thielemans, Francois [22]
572

573 **Table 2** Average (± SD) mean retention times (MRTs) in proboscis monkeys (*Nasalis*
574 *larvatus*), as calculated on the basis of two different algebraic approaches and two different
575 sampling interval frequencies for four different passage markers
576

Equation	Sampling regime*	---------- MRT ----------			
		Co (solute)	Cr (2mm)	La (5mm)	Ce (8mm)
		h			
Thielemans, Francois [22]	intensive	41.2 ±5.3	41.2 ±5.3	40.3 ±5.4	39.9 ±4.9
Thielemans, Francois [22]	less frequent	40.8 ±5.4	40.4 ±4.7	39.9 ±5.2	39.7 ±4.7
Warner [27]	intensive	32.2 ±6.3	32.9 ±6.3	32.0 ±6.2	31.6 ±5.8
Warner [27]	less frequent	37.6 ±4.3	37.1 ±3.5	36.9 ±4.0	36.7 ±3.5

577 *the 'less frequent' sampling regime was simulated by combining the results of subsequent individual samples from the
578 frequent sampling regime into 'pooled' samples, as described in Methods, resulting in a reduced number of samples entered
579 into the calculation
580

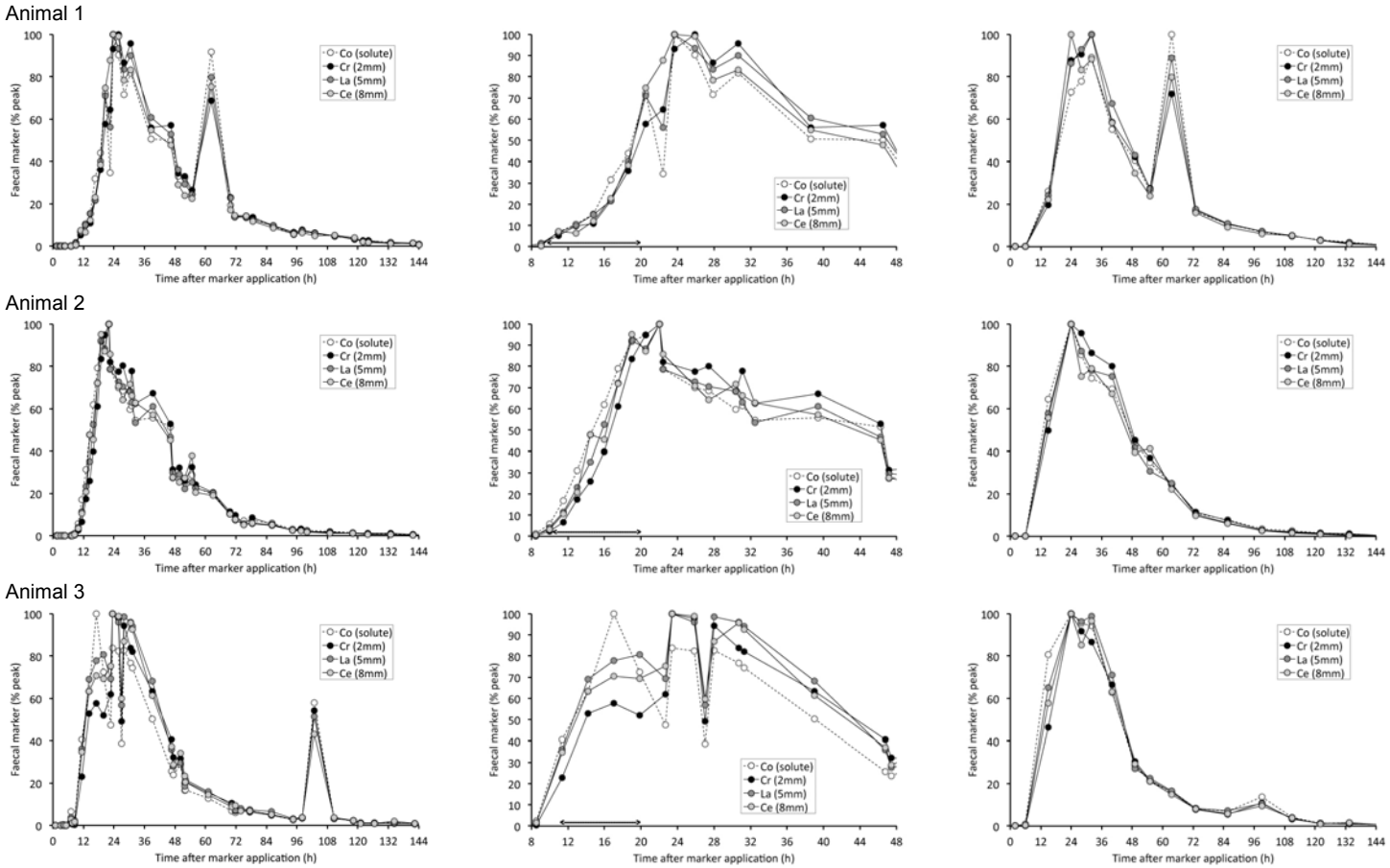


Figure 1 Passage marker (solute: 2, 5 and 8 mm particles) excretion patterns in proboscis monkeys (*Nasalis larvatus*); left column: intensive sampling regime with individual defecations collected during the first night; middle column: detailed aspect of the 8–48 h window, with the first night (from which individual samples were sorted on the basis of their marker concentration) indicated by arrows; right column: less intensive sampling regime, treating night samples as one defecation and using standardized sampling intervals by pooling individual samples taken during more frequent samplings.

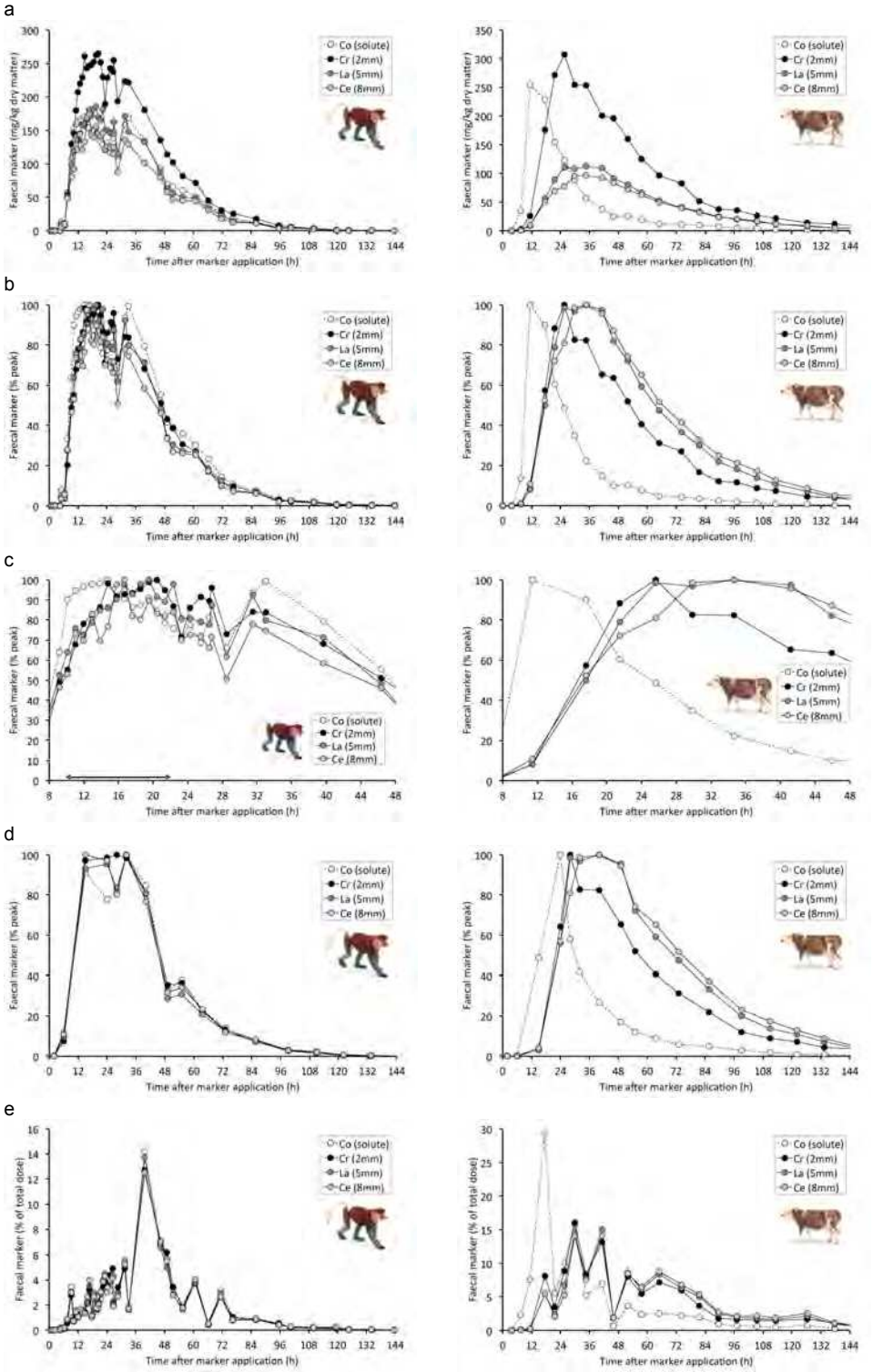
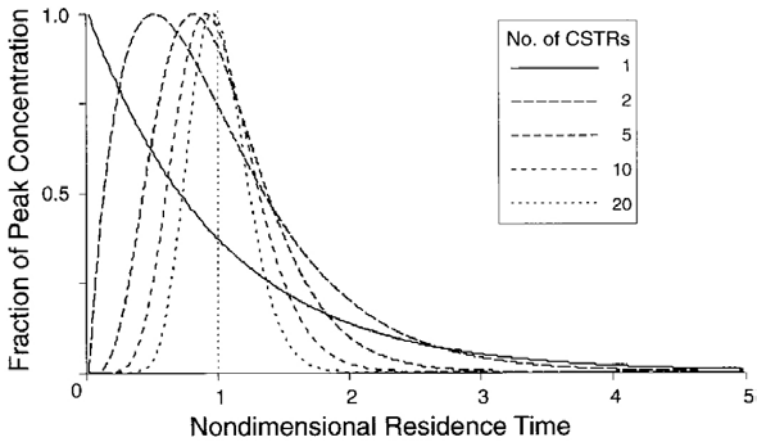
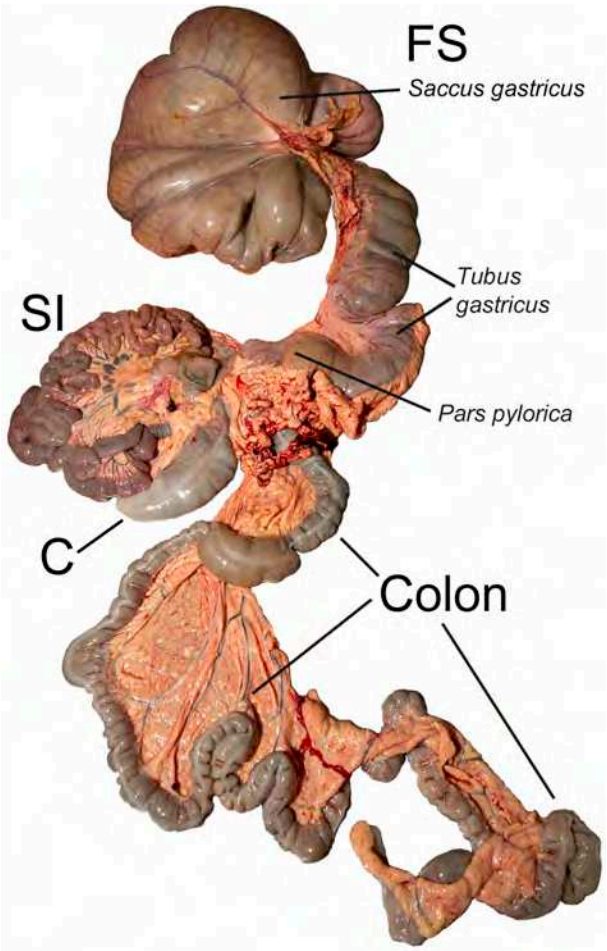


Figure 2 Passage marker (solute: 2, 5 and 8 mm particles) excretion patterns in a proboscis monkey (*Nasalis larvatus*; left column, animal 4) and a domestic heifer (*Bos primigenius taurus*; right column) in various visualizations: a) fecal concentrations (note differences between markers due to different marker dosages); b) fecal concentrations expressed as a percentage of the highest marker peak, to standardize curves between markers; c) marker concentrations, time window of 8–48 h only (arrow in proboscis indicates fecal samples from the first night, whose sequence was unknown and hence decided on the basis of marker concentrations); d) marker concentrations, treating all night samples as one defecation and using standardized sampling intervals, i.e. pooling individual samples taken during more frequent samplings; e) marker depicted as a percentage of the total dose (note the drastic difference from the other marker patterns, with a distinct pattern of consecutive peaks).



597
598 **Figure 3** Schematic representation of a marker excretion curve from chemical reactor models
599 that include various numbers of continuously stirred tank reactors (CSTRs) in series (Jumars
600 2000). Note that the y-axis contains information on the marker concentration in the outflow.
601

602



603 **Figure 4** Gastrointestinal tract of a proboscis monkey (*Nasalis larvatus*). (FS) forestomach (consisting
604 of *Saccus gastricus* and the first part of *Tubus gastricus*); the glandular stomach is represented by the
605 second part of *Tubus gastricus* and *Pars pylorica*; (SI) small intestine; (C) caecum. Nomenclature
606 from Langer (1988) and Caton (1999). Note the haustrated caecum and colon, a feature that sets
607 colobine monkeys apart from other mammalian foregut fermenters. Photograph by Warner Jens.